Arch. Biol. Sci., Belgrade, 63 (3), 747-756, 2011 DOI:10.2298/ABS1103747A

PURIFICATION AND CHARACTERIZATION OF ACID PHOSPHATASE FROM A GERMINATING BLACK GRAM (*VIGNA MUNGO* L.) SEEDLING

A.K.M. ASADUZZAMAN, M. HABIBUR RAHMAN and TANZIMA YEASMIN

Department of Biochemistry and Molecular Biology, University of Rajshahi, 6205 Rajshahi, Bangladesh

Abstract - An acid phosphatase has been isolated and purified from an extract of a germinating black gram seedling. The method was accomplished by gel filtration of a germinating black gram seedling crude extract on sephadex G-75 followed by ion exchange chromatography on DEAE cellulose. The acid phosphatase gave a single band on SDS-polyacrylamide slab gel electrophoresis. The molecular weight of the acid phosphatase determined by SDS-polyacrylamide slab gel electrophoresis was estimated to be 25 kDa. The purified enzyme showed maximum activity at pH 5 and at temperature of 55°C. Mg^{2+}, Zn^{2+} and EDTA had an inhibitory effect on the activity of the acid phosphatase. Black gram seedling acid phosphatase was activated by K^{+}, Cu^{2+} and Ba^{2+}. The Km value of the enzyme was found to be 0.49 mM for pNPP as substrate.

Key words: Germination, seedling, *Vigna mungo* L, acid phosphatase, black gram, purification, characterization and Km value

UDC 582.736.3:581.1:577

INTRODUCTION

Acid phosphatases (orthoposphoric monoester phosphohydrolase, EC 3.1.3.2) are widely distributed in plants and animals. Acid phosphatases have been purified and characterized from tubers (Kamenan, 1984; Gellatly et al., 1994; Kusudo et al., 2003; Kouadio, 2004), seeds (Ullah and Gibson, 1988; Olczak et al., 1997; Granjeiro et al., 1999), roots (Panara et al., 1990), leaves (Staswick et al., 1994), bulbs (Guo and Pesacreth, 1997) and seedlings (Yenigum and Guvenilir, 2003). Acid phosphatases catalytically break down a wide variety of phosphate esters and exhibit pH optima below 6.0 (Vincent et al., 1992). In plant roots, acid phosphatases seem to be involved in the solubilization of macromolecular organic phosphates in soils which can then be utilized by plants (Panara et al., 1990). From tubers, Kamenan (1984) and Kouadio (2004) have reported an important role of acid phosphatases in the transport of phosphate in the metabolic phenomena taking place during the preservation of yam (*Dioscorea cayenensis rotundala*) and cocoyam (*Xanthosoma sp.*) tubers.

Acid phosphatase is believed to be important for many physiological processes, including regulation of phosphorus efficiency. Exuded and internal acid phosphatase activities change in response to phosphorus availability. Low phosphorus availability increases acid phosphatase secretion to the rhizosphere in a number of plant species, including maize (*Zea mays*), tomato (*Lycopersicon esculentum)* wheat (*Triticum aestivum*) and clover (*Trifolium* spp.), lupin (*Lupinus* spp.), rice (*Oryza sativa*), and soybean (*Glycine max*). From seeds and seedlings, the physiological function of the acid phosphatases is to provide inorganic phosphate to the growing plant during germination, and many different phosphate esters of sugars and substrates stored in the seed and seedling need to be hydrolyzed during germination and growth (Gahan and McLean, 1969; Schultz and

Jensen, 1981; Akiyama and Suzuki, 1981; Hoehamer et al., 2005).

In this study, different chromatographic methods were employed to purify acid phosphatase from black gram seedlings, and their properties were examined.

MATERIALS AND METHODS

Collection of black gram seeds

Black gram seeds were collected from Bangladesh Agriculture Research Institute (BARI), sub-station of Iswardi, Pabna, and brought to the protein and enzyme laboratory of Biochemistry and Molecular Biology, University of Rajshahi. After collection, the seeds were cleaned, dried in sunlight and stored in airtight desiccators in a polyethylene bag for experimental purpose.

Black gram seed germination

Black gram seeds were sterilized with 1% (V/V) sodium hypochloride solution for 10 min, washed with water three times and soaked in water for 24 h. After hydration, germination of black gram seed was performed on a moist cotton cloth during 5 days in a room at ambient temperature of 28°C with a relative humidity of 85%. Germinating seeds were watered daily. The seedling was separated to cotyledons for enzyme activity assays.

Preparation of crude enzyme extract

In order to purify enzyme from the seedling of black gram in a biologically active form, all the operations were performed at 4°C. 50 gm of black gram was put in a mortar and ground uniformly into a fine paste. The paste was mixed uniformly with pre-cooled citrate buffer, pH 4.8 in a homogenizer and homogenized uniformly. The extract was then transferred into a beaker and kept overnight at 4°C with occasional gentle stirring. The homogenate was then filtered through a muslin cloth. The filtrate was collected and clarified further by centrifugation at 8×10^3 r.p.m. for 30 min at 4°C. After centrifugation, the clear supernatant was used as crude extract containing the partially purified that was precipitated with ammonium sulfate.

Enzyme assay

Acid phosphatase activity was based on conversion of para-nitrophenyl phosphate (pNPP) into p-nitrophenol (pNP).The reaction mixture was incubated at 37^0C for 10 min, then 2 ml of sodium carbonate (2% w/v) were added to stop the reaction and absorbances were measured at 410 nm using a spectrophotometer. pNP was used as standard. Under experimental conditions, one unit of enzyme activity was defined as 1μmol of pNP released per min. Specific activity was defined as the units of enzyme activity per mg of protein.

Buffer selection

After initial supernatant and 70% $(NH_4)_2\ SO_4$ saturation by a different buffer (100 mM sodium acetate buffer, 20 mM citrate buffer and water fraction), the highest activity of acid phosphatase was found in citrate buffer (pH 4.8). This buffer was selected for the purification step.

Protein estimation

Protein elution profiles from chromatographic columns were monitored by measuring fractional absorbance at 280 nm. The concentration of purified enzyme was determined according to Lowry et al. (1951). BSA was used as the standard protein.

Gel filtration

After dialysis with 20mM citrate buffer, the sample at pH 4.8 was loaded on a Sephadex G-75 gel column which was equilibrated with the same buffer. The column was washed with 20 mM citrate buffer, pH 4.8, containing 1 M sodium chloride.

DEAE-cellulose chromatography

The enzymatically active protein fractions obtained

after gel filtration were dialyzed with distilled water for 12 h and against 20 mM Tris-HCl buffer, pH 7.4 overnight. After centrifugation, the clear supernatant was applied to the DEAE-cellulose column which was previously equilibrated with the same buffer. The protein was eluted stepwise with increasing concentrations of the NaCl from 0 to 0.2 M with the same buffer.

Molecular weight determination by gel filtration

The molecular weight of black gram seedling acid phosphatase was determined by gel filtration on Sephadex G-75 column (90x0.9 cm^2). Lysozyme (MW 14,600), Trypsin Inhibitor (MW 20, 000) Egg albumin (MW 45,000), Bovine Serum Albumin (MW 66,000) and galactosidase (MW 116,000) were used as marker proteins as described by Andrews.

Molecular weight determination by SDS-PAGE

Proteins were analyzed by sodium dodecyl-sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli.

Effects of pH and temperature

The effect of pH on the enzyme activity was determined by measuring the hydrolysis of the substrate pNPP in a series of buffer at various pH values ranging from pH 3.0-9.0. 20 mM citrate buffer was used. The pH value of buffer was determined at 37^0C.

The effect of temperature on acid phosphatase activity was performed in 20 mM citrate buffer pH 5.0 over a temperature range of 10-90^0C using pNPP under standard test conditions.

Effect of incubation period on the activity of acid phosphatase

In order to ascertain the effect of the incubation period on the enzyme activity, the enzyme solutions were kept at 55°C, pH 5.0 for 20, 40, 60, 80 and 120 min, respectively, and the residual activity was assayed.

Effect of some chemical agents

To determine the effect of various compounds (metal ions, detergents and dithiol-reducing agents) as possible activators or inhibitors of the purified acid phosphatases, the enzymatic solutions were preincubated at 37^0C for 30 min with the compounds and then the activity was assayed. The substrate pNPP was added to the medium and incubated at 37^0C for 10 min.

Kinetic parameter of acid phosphatase

The initial velocity was equal to the amount of product formed per unit time. The initial velocity (Vi) was determined by quantitatively measuring the amount of one of the products at various time.

Substrate specificity of acid phosphatase

The substrate specificity of black gram acid phosphatase was measured using the following substrates: pNPP, phenylphosphate, galactose-1-phosphate, glucose-1-phosphate. In order to determine substrate specificity, 0.25 ml of 20 mM citrate buffer substrate pH-5.0 placed in different test tubes. Then 0.1 ml of enzyme solution was added to each tube and incubated at 37°C for 30 min, and the acid phosphatase was stopped by adding 4 ml of 0.1 N NaOH. The hydrolysis of these substrates was determined by titration of inorganic phosphate according to method.

Chemicals

Acrylamide, magnesium chloride, SDS were purchased from Sigma Chemicals Ltd., USA. DEAE-cellulose was purchased from Pharmacia Chemicals Ltd., Sweden. All other chemicals were analytical grade.

RESULTS AND DISCUSSION

Acid phosphatase activity in germinating black gram seedlings

During the germinating stage the acid phosphatase activity in black gram ranged from 0.08-4.9 unit/L.

The highest activity of acid phosphatase was found after 70% $(NH_4)_2 SO_4$ saturation (Table-1).

Buffer selection

Enzymatic activity of acid phosphatase from black gram seedlings by different buffer is shown in Fig. 1.

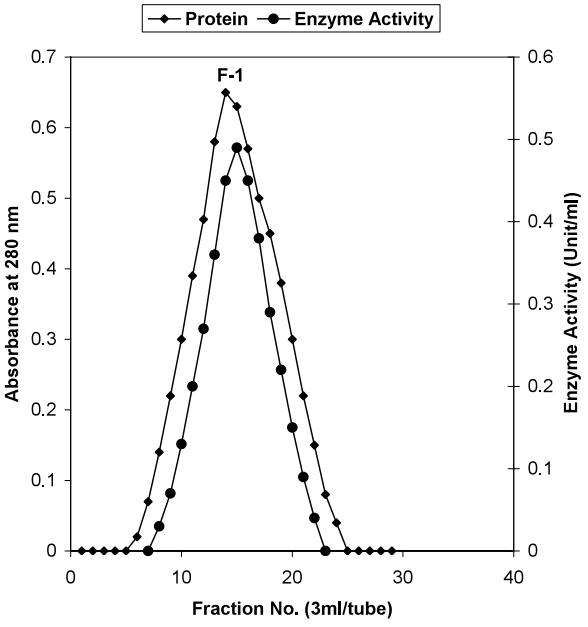

Fig. 1. Elution profile of 70% ammonium sulfate saturated crude extract of germinating black gram seedling on sephadex G-75 column.
Size of column: 2.5 × 100 cm
Buffer: 20 mM citrate buffer

Gel filtration chromatography

The crude protein extract was eluted as two main peaks, namely F-1 and F-2. It was found that the F-1 fraction contained acid phosphatase activity while the F-2 peak showed no activity (Fig. 2). The fraction F-1 containing the acid phosphatase activity as indicated by a solid line was pooled, concentrated and further purified by DEAE-cellulose column chromatography.

DEAE-cellulose chromatography of F-1 fraction

The components of the F-1 fraction were separated into four peaks F-1a, F-1b, F-1c and F-1d, with the buffer containing 0.02 M, 0.05 M, 0.1 M and 0.2 M NaCl gradients, respectively. The enzymatic activity of all these fractions was investigated and it was found that the fractions from peak F-1c contained acid phosphatase activity while the fractions F-1a, F-1b and F-1d possessed no acid phosphatase activ-

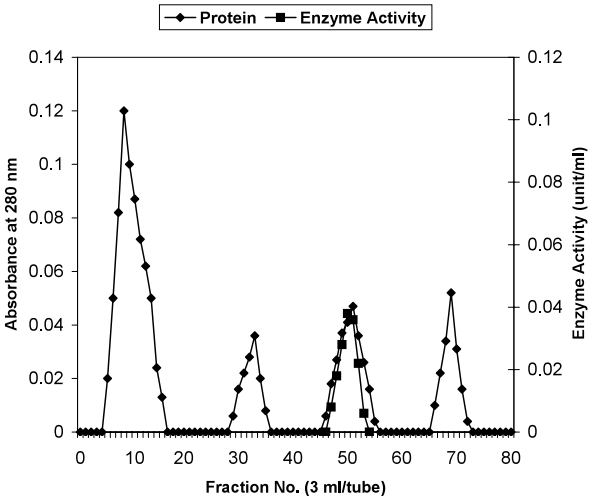

Fig. 2. Elution profile of F-1 fraction acid phosphatase on DEAE-cellulose column.
Size of column: 2.2 × 15 cm
Buffer: 20 mM Tris-HCl buffer

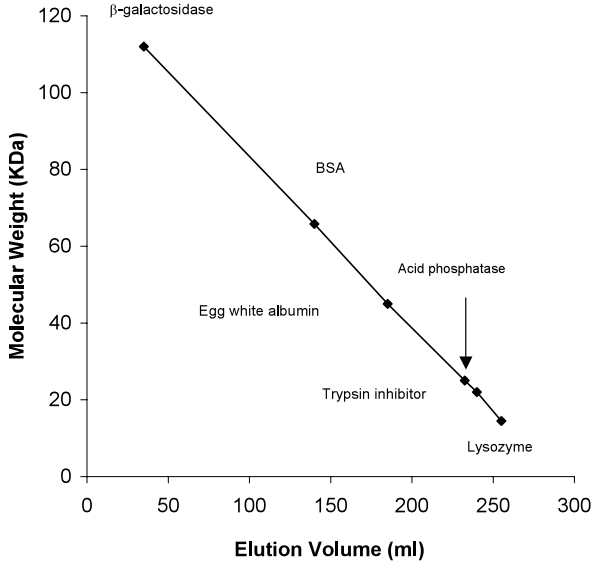

Fig. 3. Standard curve for the determination of molecular weight of acid phosphatase by gel filtration method

ity (Fig. 3). The fraction containing acid phosphatase activity was pooled separately and dialyzed against 20 mM Tris-HCl buffer, pH 7.4 at 4°C overnight, and then concentrated.

Determination of molecular weight by gel filtration method

The molecular weight of the enzyme was determined by comparing their elution volume on sephadex G-75 with those of the marker proteins under the same experimental conditions. The molecular weight was calculated from the standard curve (Fig. 4) of the reference proteins, constructed by plotting a log of molecular weight against elution volume on gel filtration. The molecular weight of purified enzyme was found to be 25 kDa.

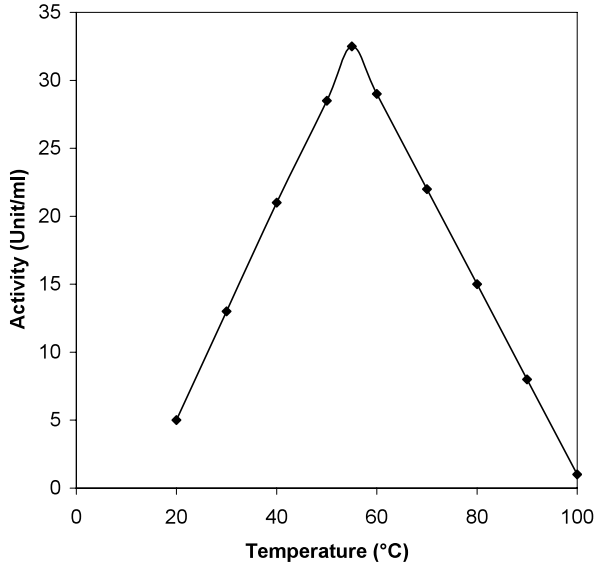

Fig. 4. Effect of temperature on the activity of acid phosphatase from the germinating black gram seedling

Molecular weight determination by SDS-PAGE

The molecular weight of the enzyme was also determined by SDS slab gel electrophoresis (SDS-PAGE) method (plate-1) using β-galactosidase (MW 116,000), Bovine Serum Albumin (MW 66,000), Egg white Albumin (MW 45,000), Trypsin Inhibitor (MW 20,000), Lysozyme (MW 14,600) as standard proteins. The molecular weight of purified enzyme was found to be 25 kDa.

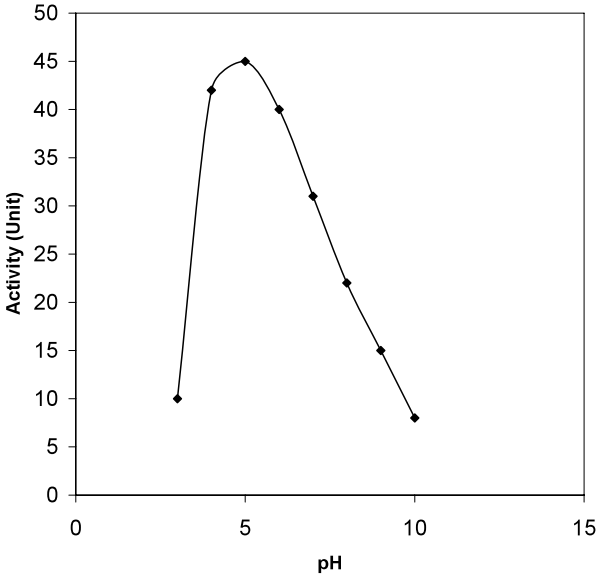

Fig. 5. Effect of pH on the activity of acid phosphatase from the germinating black gram seedling

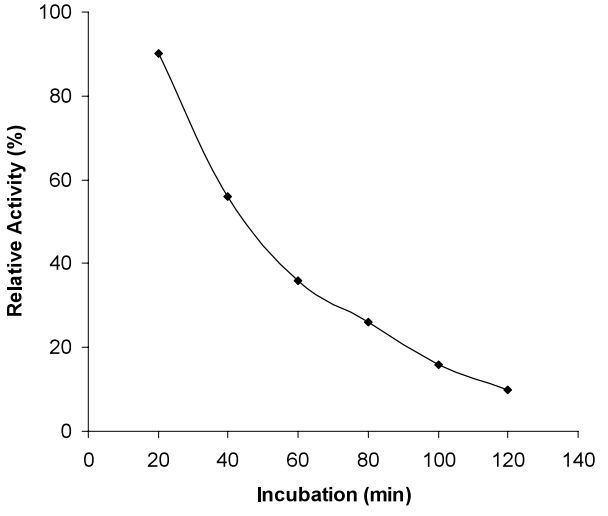

Fig. 6. Effect of incubation time at optimal temperature on the acid phosphatase activity of germinating black gram seed

Effects of pH and temperature

The effect of the pH and temperature on acid phosphatase activity is shown in Figs 5 and 6. The enzyme

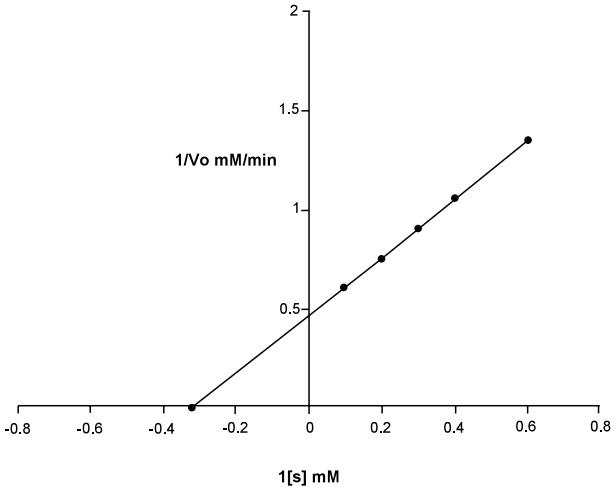

Fig. 7. Lineweaver-Burk double reciprocal plots for the determination of Km value of the germinating black gram seedling acid phosphatase

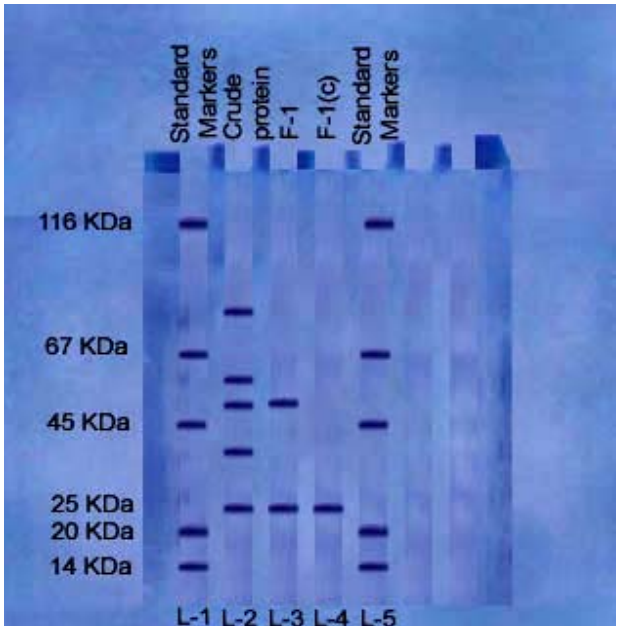

Fig. 8. 10% SDS slab gel electrophoretic pattern of the purified enzyme for determination of molecular weight
L-1 & L-5: β-galactosidase (MW 116,000), Bovin Serum Albumin (MW 66,000), Egg white Albumin (MW 45,000), Trypsin Inhibitor (MW 20,000), Lysozyme (MW 14,600)
L-2: Crude enzyme solution. L-3: Faction from gel filtration chromatography. L-4: Faction from DEAE-cellulose column

was most active at pH 5.0 and 55^{0}C. Above 55^{0}C, the enzyme activity declined rapidly as the temperature increased, but the enzyme was not completely inactivated at 80^{0}C.

Determination of incubation period

Purified acid phosphatase activity at various incubation periods is shown in Fig. 7. Incubation at the assay temperature for acid phosphatase activity (55°C) for various incubation periods showed that the enzyme gradually loses with time. The highest acid phosphatase activity was found when the homogenate was assayed after heat treatment for 30 min. After 1 h or even 2 h, the activity of the acid phosphatase remained at 44%. The decrease in activity with time was probably due to denaturation of the enzyme or the self-degradation properties of enzyme.

Effect of metal ions and chelating agents

The influence of various metal ions and chelating agents on the purified acid phosphatase is presented in Tables 2 and 3. Black gram seedling acid phosphatase was activated by K^+, Cu^{2+} and Ba^{2+}, while Mg^{2+}, Sr^{2+} and Zn^{2+} had an inhibitory effect on the activity of acid phosphatase. EDTA, a metal chelating agent, had an inhibiting effect on the acid phosphatase of the seedlings of black gram .The decrease in activity may be due to the removal of metal ions located on or near the active site.

Kinetic properties of acid phosphatase

The activity of purified acid phosphatase was analyzed using different concentrations of para-nitrophenyl phosphate as substrate. As shown in Fig. 8, the extrapolated Km value for acid phosphatase from black gram seedlings was estimated to be 0.49 mM.

Substrate specificity of acid phosphatase

Activity of the acid phosphatase was assessed using the different phosphate compounds as substrate and the result is presented in Table 4. The acid phosphatase was found to be capable of hydrolyzing pNPP, phenyl

Table 1. Acid phosphatase activity in germinating black gram seedling

Pulse	Code	Fraction	Enzyme activity (Units/L)
Black gram (Mashka-lai) *vigna mungo* L.)	SN1	20,000×g sup.	2.05
	P1	20,000×g sed.	0.08
	SN2	40%AS sup.	1.27
	P2	40%AS sed.	1.83
	SN3	70%AS sup.	1.06
	P3	70%AS sed.	4.46
	SN4	Sup. of diss. P3	4.9

Table. 2. Effect of EDTA on the activity of acid phosphatase enzyme

Concentration of EDTA (molar)	**Relative activity (%) Acid phosphatase**
0.000	100
0.001	76.5
0.002	62.5
0.005	40
0.010	25
0.020	19

Table 3. Effect of various metallic salts on acid phosphatase activity

Concentration of metal salts (molar)		**Relative acid phosphatase activity (%)**
None		**100**
$MgCl_2$	0.001	93.4
	0.005	66.5
$BaCl_2$	0.001	156.2
	0.005	223.3
KCl	0.001	125
	0.005	127.3
$ZnCl_2$	0.001	64.8
	0.005	19
$FeCl_3$	0.001	113.2
	0.005	129
NaCl	0.001	99
	0.005	110
$SrCl_2$	0.001	197
	0.005	193
$CuCl_2$	0.001	120.23
	0.005	190

phosphate and glucose-1- phosphate. The hydrolysis activities of these three substrates varied from 21.4 to 100 for the respective acid phosphatase. But the enzyme had no effect on galactose-1-phosphatase.This indicated that the activity of the enzyme was not restricted to a single substrate.

CONCLUSION

Germination is accompanied by the synthesis or activation of enzymes responsible for the degradation of seed reserves. Among these enzymes, acid phosphatases are involved in the metabolic proc-

Table- 4: Substrate specificity of acid phosphatase

Substrates	Hydrolysis activity %
p-Nitrophenylphosphate	100
Phenylphosphate	93.10
Glucose-1-phosphate	21.4
Galactose-1-phosphate	0

Table-5: Acid phosphatase purification from germinating black gram seedling

Fraction	Total protein (mg)	Total Activity	Specific activity (Unit/mg)	Yield (%)	Purification Fold
Crude enzyme	190	590	3.1	100	1
70% $(NH_4)_2SO_4$ saturated	47	405	8.62	68.64	2.78
After gel filtration (F-1)	8.16	385	47.18	65.25	15.22
DEAE cellulose column chromatography	0.95	129	135.78	21.86	43.8

esses of germination and maturation of plants. They are constitutively expressed in seeds during germination, and their activities increase with germination to release the reserve materials for the growing embryo (Biwas and Cundiff, 1991; Thomas, 1993). In the present study, acid phosphatase activities increased and reached the maximum on the 5th day of black gram seeds germination at temperatures below 28^{0}C. Prazeres et al.(2004) reported that when pNPP was used as a substrate the maximum acid phosphatase activity of soybean seedlings was detected on the 6th and 9th days, for germination temperatures of 28^{0}C and 20^{0}C, respectively. These results suggest that temperature and seed species have an influence on germination phenomena.

Several studies have been devoted to the purification of acid phosphatases from the cotyledons of germinating seeds (Ullah and Gibson, 1988; Basha, 1984; Granjeiro et al., 1999). However, few works concerning the purification of seedlings' acid phosphatase have been reported so far (Bhargava and Sachar, 1987; Yenigum and Guvenilir, 2003). To better understand the role played by acid phosphatase during black gram seed germination, acid phosphatases from the crude extract of black gram seedlings were purified to homogeneity by chromatographic process and their properties were examined.

Acid phosphatase enzyme was purified from a germinating black gram seedling with the molecular weight of about 25 KDa. Black gram seedling acid phosphatase was close to that of peanut seedlings (22.4 &24.0 kDa, respectively) (Gonnety et al., 2006) and were lower than that of the potato tuber (100 kDa) (Gellatly et al., 1994), peanut seed cotyledons (240 kDa) (Basha, 1984). Tomato cell culture (92 kDa) (Paul et al., 1987), barley roots (79 kDa) (Panara et al., 1990), wheat seedlings (35 kDa) (Chen and Tao, 1989) and soybean seeds (51, 58, 52 and 30 kDa) (Ferreira et al., 1998). However, black gram seedling acid phosphatases had a higher molecular weight than the purified acid phosphatase from bovine heart (18 kDa) (Zhang and Van-Etten, 1990).

The enzyme showed maximum activity at pH 5 and temperature 55°C. The optimum pH determined for the black gram seedling acid phosphatases was largely consistent with other pH optima (5.0-6.0) (Ferreira et al., 1998; Duff et al., 1989; Hass et al., 1991; Gonnety et al., 2006). However, acid phosphatases from peanut seedlings and soybean seeds

exhibited maximum catalytic activity at 55^{0}C and 60^{0}C (Gonnety et al., 2006 & Ullah and Gibson, 1988).

The purified acid phosphatase was sensitive to ions at various degrees depending on the ions' nature and isoenzymes. The requirement of metallic ions for acid phosphatase activity has also varied according to plant species, development stage and isoenzymes heterogeneity (Scandalois, 1974; Panara et al., 1990; Tso and Chen, 1997). However, Mg^{2+}, Zn^{2+} and EDTA were inhibitors and, K^{+}, Cu^{2+} and Ba^{2+} were activators for the enzyme. The inhibition of acid phosphatase activity by Zn^{2+} has been previously reported in other plant species such as pea (Mizuta and Suda, 1980), tobacco (Pan et al., 1987) and peanut seedlings (Gonnety et al., 2006).

For the enzymes, kinetic study correlates well with substrate specificity and showed a Lineweaver-Burk plot. The purified acid phosphatase showed the highest catalytic efficiency toward pNPP. The Km value (0.49 mM) of acid phosphatase was similar to the Km value of acid phosphatase from soybean seeds and peanut seedlings toward pNPP (Ferreira et al., 1998; Gonnety et al., 2006). The purified enzymes hydrolyzed broad phosphorylated substrates to various degrees. Similar observations have been reported for the acid phosphatase from sweet potato (Kusudo et al., 2003) and peanut seedlings (Gonnety et al., 2006). This indicates that the activity of enzyme was not restricted towards a single substrate.

REFERENCES

Akiyama, T. and *H. Suzuki* (1981). Localization of acid phosphatases in aleurone layers of wheat seeds. *Pfanzenphysiol*. **101**, 131.

Andrews, P. (1965). The gel filtration behavior of proteins related to their molecular weights over a wide range. *Biochem. J.* **96**, 595-605.

Basha, S.M. (1984). Purification and characterization of an acid phosphatase from peanut (*Arachis hypogaea*) seed. *Can. J. Bot*. **62**, 385-1297.

Bhargava, R., and *R.C. Sachar* (1987). Identification of acid phosphatase in cotton seedlings: Purification, subunit structure and kinetic properties. *Phytochem*. **26**, 1293-1297.

Bieleski, R. (1973) Phosphate pools, phosphate transport, and phosphate availability. *Ann Rev Plant Physiol*, **24**, 225-252.

Biwas, T.K., and *C. Cundiff* (1991). Multiple forms of acid phosphatases in germination seeds of *vigna sinensis, Phytochem*. **30**, 2119-2125.

Chen, H.F., and *M. Tao* (1989). Purification and characterization of a phosphotyrosyl-protein phosphatase from wheat seedlings. *Biochim. Biophys. Acta* 19:271-276.

Clark, R.B. (1975) characterization of phosphatase of intact maize roots. *J Agri Food Chem*. **23**, 458-460.

Duff, S.M.G., Lefebvre, D.D., and *W. Plaxton* (1989). Purification and characterization of a phosphoenonol pyruvate phosphatase from Brassica nigra suspensions cells. *Plant physiol*. **90**, 734-741.

Duff, S.M.G., Sarath, G., and *W.C. Plaxton* (1994) The role of acid phosphatases in plant phosphorus metabolism. *Physiologia Plantarum* **90**, 791-800. [ISI].

Ferreira, C.V., Granjeiro, J.M., Taga, E.M., and *H. Aoyama* (1998). Purification and characterization of a phosphoenonol pyruvate phosphatase from Brassica nigra suspension cells. *Plant Physiol. Biochem*. 36: 487-494.

Gahan, P.B., and *J. McLean* (1969). Subcellular localization and possible functions of acid β-glycerophosphatase and naphthol esterases in plant cells. *Planta*. **89**, 126-136.

Gellatly, K.S., Moorehead, G.B.G., Duff, S.M.G., Lefebvre, D.D., and *W. Plaxton,* (1994). Purification and characterization of potato tuber acid phosphatase having significant phosphotyrosine activity. *Plant physiol*. **106**, 223-232.

Goldstein, A., Baertlein, D., and *R. McDaniel* (1988a). Phosphate starvation inducible metabolism in Lycopersicon esculentum: I. Excretion of acid phosphatase by tomato plants and suspension cultured cells. *Plant physiol*, **87**, 711-715.

Goldstein, A., Danon, A., Baertlein, D., and *R. McDaniel* (1988b) Phosphate starvation inducible metabolism in Lycopersicon esculentum: II. Characterization of the phosphate starvation inducible excreted acid phosphatase. *Plant physiol*. **87**, 716-720.

Gonnety, J.T., and *L.P. Kouame* (2006). Purification and characterization of three low-molecular weight acid phosphatase from peanut (*Arachis hypogaea*) seedlings. *AJB* Vol. 5 (10, pp. 035-044.

Granjeiro, P.A, Ferreira, C.V, Taga, E.M., and *H. Aoyama* (1999). Purification and kinetic properties of a castor bean seed acid phosphatase containing sulfhydryl groups. *Plant physiol.* **107**,151-158.

Guo, J., and *J.C. Pesacreta* (1997). Purification and characterization of an acid phosphatase from the bulb of *allium cepa* L. *J. Plant physiol*. 151: 520-527.

Hass, H., Redi, B., Leitner, E., and *G. Stoffler* (1991). Penicillium Chrysogenum extracellular acid phosphatase; purification and biochemical characterization. *Biochem. Biophys. Acta* **1074**, 392-397.

Hoehamer, C.F., Mazur, C.S., and *N.L. Wolfe* (2005). Purification and partial characterization of an acid phosphatase from *spirodela oligorrhiza* and its affinity for selected organophosphate pesticides. *J. Agrc. Food Chem*. **53**, 90-97.

Kamenan, A. (1984). Purification et etude physico-chimique de trios phosphatases acids de *Dioscorea cayenensis rotundata*. These de doctorat d'Etat es- sciences physiques Faculte des sciences de l'Universite d'Abidjan Cocody, Cote d'Ivoire. pp. 134.

Kouadio, N.E.J.P (2004). Contribution a l'etude du tubercule de *Taro Xanthosoma* sp variete atoumbou orono: evolution de quelques parameters biochimiques au cours de la conservation et purification et caracterisation de deux phosphatases acides. These de doctorat 3$^{\theta me}$ cycle de l'UFR des sciences et technologie des aliments de l' Universite d'Abobo-Adjame (Cote d'Ivoire). 10 pp.

Kusudo, T., Sakaki, T., and *K. Inouye* (2003). Purification and characterization of purple acid phosphatase P1 from from dry power of sweet Potato. *Biosci. Biotechnol. Biochem*. **67**, 1609-1611.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage Tu. *Nature*. **227**, 680-685.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L and *R.J. Rendall* (1951). Protein measurement with the Folin Phenol reagent, *Biol. Chem*. **183**, 265-275.

Mizuta, S., and *S. Suda* (1980). A comparative study of multiple acid phosphatases in cellular fractions of bean hypocotyls. *Ann. Bot*. **45**, 369-362.

Olczak, M., Watorek, W., and *B. Morawieka* (1997). Purification and characterization of acid phosphatase from yellow lupin (*Lupinus luteus*) seeds. *Biochim. Biophys. Acta*, **1341**, 14-25.

Pan, S.M., Huang, Y.F., and *Y.R. Chen* (1987). Characterization and localization of acid phosphatase in the suspension cultured cells of N*icotiana otophora*. *Taiwania*. **32**, 1-10.

Panara, F., Pasqualini, S., and *M. Antonielli* (1990). Multiple forms of barley root acid phosphatase: Purification and some characteristics of major cytoplasmic isoenzyme. *Biochim. Biophys. Acta*. **1037**, 73-80.

Paul, E.M., and *V.M. Williamson* (1987). Purification of acid phosphatase-1 from a nematode- resistant tomato cultivar. *Plant physiol*. **84**, 399-403.

Prazeres, J.N., Ferreira, C.V., and *H. Aoyama* (2004). Acid phosphatase activities during the germination of *glycine max* seeds. Plant Physiol. Biochem. **42**, 15-20

Robyt, J.F. and *B.J. White* (1990). Biochemical Techniques. Theory and practice (Book). pp. 295-296 and 304-305.

Scandalois, G. (1974). Isozyme in development and differentiation. *Annu. Rev. Plant Physiol*. **25**, 225-228.

Schultz, P., and *W.A. Jensen* (1981). Pre-fertilization ovule development in capsella: ultrastructure and ultracytochemical localization of acid phosphatase in the meiocyte. *Protoplasma* **107**, 27-45.

Staswick, P.E., Papa. C., Hung, J., and *Y. Rhee* (1994). Purification of major soybean leaf acid phosphatase that is increased by seed-pod removal. *Plant Physiol*. **104**, 49-57.

Tadano, T., Ozawa, K., Sakai, H., Osaki, M., and *H. Matsui* (1993) Secretion of acid phosphatase by the roots of crop plants under phosphorus deficient conditions and some properties of the enzyme secreted by lupin roots. *Plant Soil.* **156**, 95-98.

Tarafder, J.C, and *K. A. Jung* (1987) Phosphatase activity in the rhizosphere and its relation to the depletion o soil organic phosphorus. *Biol Fertil Soils*. **3**, 199-204.

Taussky, H.H., and *E. Shorr* (1953). A microeconometric method for determination of organic phosphorus. *J. Biol. Chem*. **2002**, 675-685.

Thomas, T.L. (1993). Gene expression during plant embryogenesis and germination: an overview plant cell. **5**, 1401-1410.

Tso, S.C., and *Y.R. Chen* (1997). Isolation and characterization of a group III isoenzyme of acid phosphatase from rice plants. *Bot. Bull. Acad. Sin*. **38**, 245-250.

Ullah, A.H., D.M. Gibson (1988). Purification and characterization of acid phosphatase from cotyledons of germinating soybean seeds. *Arch. Biochem. Biophys*. **260**, 514-520.

Vincent, J.B., Crowder, M.W., and *B.A. Averill* (1992). Hydrolysis of phosphate monoester: a biological problem with multiple chemical solutions. *Trends Biochem. Sci*. **17**, 105-110.

Yenigun, B., and *Y. Guvenilir* (2003). Partial purification and kinetic characterization of acid phosphatase from garlic seedling. *Appl. Biotechnol.* **105**, 677-687.

Zhang, Z.Y., and *R.L. Van-Etten* (1990). Purification and characterization of a low-molecular-weight acid phosphatase-A Phosphotyrosyl-protein phosphatase from bovine heart. *Arch. Biochem. Biophys*. **282**, 39-49.